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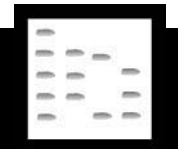
AROMAS: GAS CHROMATOGRAPHY

See **III / FRAGRANCES: GAS CHROMATOGRAPHY**

ART CONSERVATION: USE OF CHROMATOGRAPHY IN



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Introduction

Analytical science plays a vital role in the conservation of our artistic heritage and chromatography is one of the most valuable techniques available to the conservation scientist.

In order to design the optimum safe conservation/restoration treatment plan, which takes account of the nature of the original materials used by the artist, conservators require a detailed knowledge of the materials used. The microscopic samples characteristic of work in this area are notoriously problematic to deal with and the sensitivity of the analytical technique is paramount.

The question why are some painted works in better condition than others of a similar age? is an important one for the conservator and specific information regarding the nature of the media used in such works may offer some insight as to why variations in the ageing characteristics of individual paintings occur.

Paint Media

Artists have traditionally used a diverse range of materials as binding media for their pigments: natural oils, gums and proteinaceous materials such as egg, milk and collagen glues have all been incorporated into paint layers.

Oil painting was popular in northern Europe from before the 13th century and analytical evidence suggests that linseed oil was favoured, whilst in Italy, where oil painting was introduced in the 15th century, walnut oil was initially preferred. The oils most widely used in western European art are linseed, walnut and poppy, though the use of other oils, such as tung and safflower, has become more common in recent years.

Plant gums are commonly found as adhesives and binders. Gum arabic is primarily used as a painting medium, but others such as gum tragacanth (a medium for painting on linen) and cherry gum (which results in an enamel-like effect when mixed with egg or casein emulsions) are used less frequently. There is documentary evidence to suggest that gums have been employed as binding media and sizing materials for centuries: gum was used as a replacement for sun-dried oil as early as the 12th century.

Proteinaceous media include gelatine, milk and egg proteins. Animals and fish collagen glues are widely used as strong adhesives for wood, binders in the preparation of grounds, size for canvas, and pigment binders in decorative paints. Casein (a mixture of related phosphoproteins found in milk products), egg albumin (glair) and egg yolk (tempera) have traditionally found uses as pigment binders, temporary

varnishes and sealant over primers or grounds: in addition, casein provides one of the strongest natural adhesives known, much used by joiners and cabinet makers in the past.

The choice of binding medium is determined by a number of factors, predominantly the nature of the pigments being used, coupled with the effect desired by the artist, plus historical factors like location and the period of the piece. A variety of materials have enjoyed periodic popularity due to artistic trends and scientific progress.

Analysis of Paint Media

The analyses of oil-based media are well documented, but any information on the nature of other paint media has been obtained primarily via microscopic staining methods or crude solubility tests. Existing methods of analysis provide the basis for the separation of the general categories of binding media (oil, gum and protein) by qualitative means: for example, oil and protein layers can be distinguished by the differential staining of cross-sections, whilst colorimetric spot tests can be used for the identification of polysaccharide gums.

The applicability of these microanalytical techniques *in situ* can be advantageous, but the results can be misleading or inaccurate and for this reason these techniques are best used in conjunction with other analytical methods.

Such simple qualitative techniques, including paper and thin-layer chromatography (TLC), are adequate where merely the general category of binding medium is required, or where the material contains unique constituents (e.g. hydroxyproline in gelatine). However, only quantitative chromatographic techniques will enable differentiation between the similar binding media where they have no distinctive composition, such as in egg and milk proteins.

Gas Chromatography

Conservation scientists have routinely used gas chromatographic (GC) methods for the analysis of proteinaceous, oil and gum media for many years. The technique is highly sensitive – an important factor in the analysis of small samples – and a selection of derivatizing agents is available for use.

GC analysis of trimethylsilyl derivatives of amino acids in protein hydrolysate has been widely reported. The carboxyl group of an amino acid is easier to silylate than the amino group, which results in the formation of two products upon silylation: under mild conditions, the silyl ester is the major product formed using hexamethyldisilazane as the silylating agent. Silylation of the amino group usually requires a stronger donor and silylation with either *N*-trimethylsilyldiethylamine or *N*,*O*-bis(trimethylsilyl)acetamide yields the silylamine-silyl ester.

Volatile butyl ester derivatives of amino acids found in the protein hydrolysate of binding media from paint layers and priming removed from 16th- and 18th-century easel and wall paintings have been used for GC analysis. Derivatization is achieved in two stages: the carboxyl functions are first converted into butyl esters by the addition of butanol (with dissolved hydrogen chloride), then the amino groups are acetylated with trifluoroacetic anhydride. The samples, dissolved in ethyl acetate, are separated on a temperature-programmed capillary column. Calculation of the relative peak areas of each amino acid revealed a distinctive profile for each of the binding media.

The existence of an amino acid profile was established for each of the protein media types, confirming their identity by the characteristic amino acid ratios seen for each. Proteinaceous material from the gesso, ground and paint layers of a selection of Italian works was hydrolysed under acid conditions, then deionized on a small ion exchange column. The samples were then successfully methylated (carboxyl function) and acetylated (amino function), yielding the highly volatile *N*-acetylmethyl esters of the amino acids, which were separated on a packed column. Results are shown in **Table 1**.

Loss of analytes is a problem associated with acid hydrolysis. The acid hydrolysis of proteinaceous samples in the presence of carbohydrates may lead to the elimination of amino acids as humins, which cause darkening of the hydrolysate and the formation of insoluble matter. A major contributory factor in the production of humins (a mixture of coloured compounds which resemble natural melanins) is the presence of tryptophan and amino sugars (e.g. galactosamine) or carbohydrates in the sample, which degrade during acid hydrolysis.

GC has been used to quantify the fatty acid content of eggs. The use of a tempera medium can be confirmed by the absence of azelate in the presence of both palmitate and stearate nondrying oils (i.e., oils which thicken but do not dry to a skin). Samples removed from aged paint films were saponified before methylation with diazomethane, then injected directly on to a wide-bore column. The presence of the methyl esters of saturated palmitic and stearic acids, with variable amounts of unsaturated oleic acid, was revealed. This method is also applicable for the analysis of oil-based media, the palmitate : stearate ratio proving the means of differentiation.

Table 1 Percentage amino acid composition (calculated from peak areas)

	Amino acid percentage, peak areas							
	Laboratory aged samples				*Samples from paintings*			
Amino acid	*Test 1*	*Test 2*	*Test 3*	*Test 4*	*Sample 1*	*Sample 2*	*Sample 3*	*Sample 4*
Alanine	3.9	10.2	5.1	10.0	2.3	10.9	7.5	14.4
Valine	4.4	4.6	6.9	2.4	19.0	1.5	4.8	1.3
Glycine	1.5	4.1	7.4	19.4	3.3	17.5	3.3	27.9
Isoleucine	2.3	3.1	5.2	1.1	4.0	0.6	2.6	0.4
Threonine	4.1	4.9	5.4	2.2	0.1	1.4	4.2	1.4
Leucine	16.4	15.5	11.8	4.0	10.6	3.9	13.0	4.7
Serine	5.8	9.8	7.0	2.9	8.1	2.6	7.9	3.6
Proline	21.0	7.7	5.4	19.6	14.4	20.6	6.3	8.3
Aspartic acid	8.0	15.6	11.1	5.1	7.4	4.6	11.6	7.0
Hydroxyproline				14.2	0.2*	15.7		13.7
Methionine	0.4	0.1	1.1	0.3		0.0	0.2	0.2
Glutamic acid	14.9	11.3	13.2	8.6	18.1	11.6	18.1	13.1
Phenylalanine	5.4	8.0	9.1	3.9	11.8	4.1	13.6	2.8
Lysine	11.2	4.4	10.7	5.6	0.4	4.2	6.1	0.7

Test 1, casein ground; test 2, glair/chalk mix; test 3, egg yolk/chalk mix; test 4, rabbit skin glue/chalk mix; sample 1, upper red layer from the façade of San Petronio, Bologna; sample 2, gesso ground from Bellini's *The Madonna of the Meadow*; sample 3, unpigmented priming from Bellini's *The Madonna of the Meadow*; sample 4, ground layer from Baccafumi's *Tanaquil*. Reproduced from White (1984) with permission.

A mixed medium such as tempera contains both fatty acids and amino acids and GC has been employed to analyse both components simultaneously. Samples of mixed proteinaceous and oil media were hydrolysed under acid conditions, and derivatized to yield the volatile *N*-(*O*, *S*)-ethoxycarbonyl ethyl esters, which were then separated by capillary GC. This method has also been used for the analysis of amino acids alone.

Volatile ethyl chloroformate derivatives of amino acids in the hydrolysate of samples of proteinaceous media have been analysed to study the effects of pigments and ageing on the actual analysis/characterization of proteins from art objects, the results being interpreted via statistical methods.

GC is the method of choice for the analysis of natural gum media from works of art, though to date there has been relatively little work published in this area. An obvious problem associated with the use of many analytical techniques for this type of analysis is the insufficient sensitivity of the method for the microscopic samples available to the conservation scientist. However, progress has been made in the analysis of gum media by GC, often in conjunction with TLC.

Trimethylsilyl derivatives of sugars from the hydrolysed samples of surface coating taken from a wooden Egyptian sarcophagus (dating from the 21st dynasty) were analysed using a combination of GC and TLC, revealing the presence of gum tragacanth. The same procedure was also employed for the analysis of the paint medium itself and disclosed the use of a mixture of gum tragacanth and honey.

Samples of media taken from Asian wall paintings were also found to contain polysaccharide material when analysed by GC and TLC. Gum sample hydrolysates were acetylated prior to analysis and characterization of the media, but the actual method of sample preparation was lengthy and tedious, requiring 12 h for hydrolysis and a further 5 h for derivatization.

Results obtained for the GC analysis of samples of gum from trees of the *Acacia* genus growing in the vicinity of the Tomb of Nefertari revealed that they were lacking in rhamnose, which usually indicates gum tragacanth, whilst the remainder of the sugar content matched that of gum arabic from other sources. When samples of media from paintings in the tomb were analysed by GC, the same sugar profile was observed: it was therefore concluded that the paint medium was in fact gum arabic.

Twilley's comprehensive analytical studies of natural gums and their artistic applications employed a variety of techniques, including the GC analysis of trimethylsilyl sugar derivatives. In addition, GC was used for the analysis of reference samples of aged ink, thus enabling the characterization of ink samples taken from a number of ancient manuscripts.

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GC with a mass selective detector following a simple 'one-pot' hydrolysis and derivatization procedure was used for the characterization of a number of suspected gum media samples taken from the paint and ground layers of tempera works by William Blake. The results frequently indicated the presence of mixed gum media (typically comprising gums arabic, tragacanth and karaya) with the addition of cane sugar. **Figure 1** shows the chromatograms of four standard gum media samples, whilst **Figure 2** shows the chromatograms obtained for samples of priming and paint media removed from two works by Blake, *Spiritual Form of Nelson Guiding Leviathan* (1805–9) and *Body of Christ Borne to the Tomb* (1799–1800).

GC has also facilitated the analysis of coatings, such as resins, waxes, lacquers and varnishes removed from works of art. Coatings are often applied

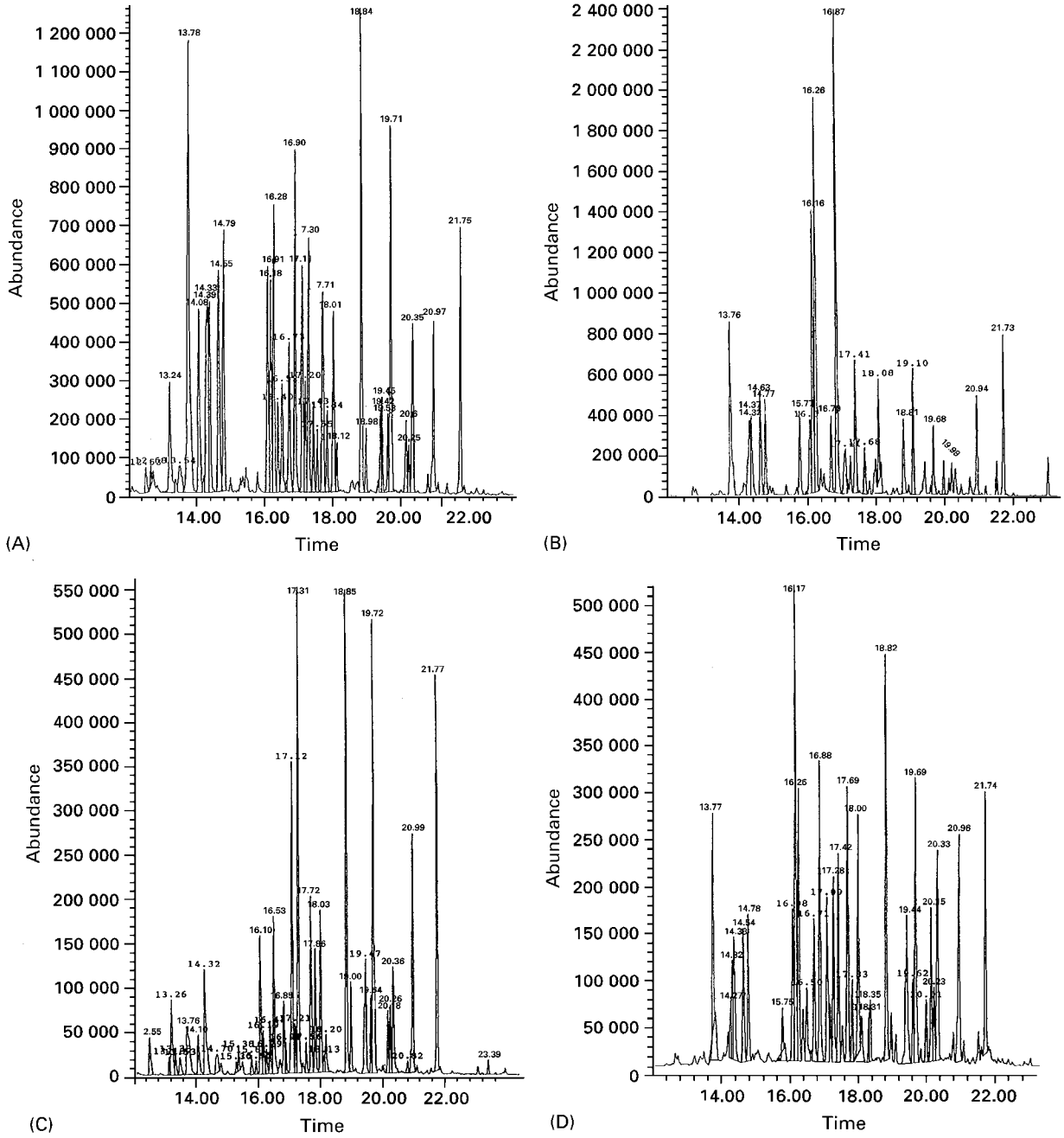

Figure 1 Chromatograms of four standard gum media samples: (A) gum arabic; (B) gum tragacanth; (C) karaya gum; (D) cherry gum.

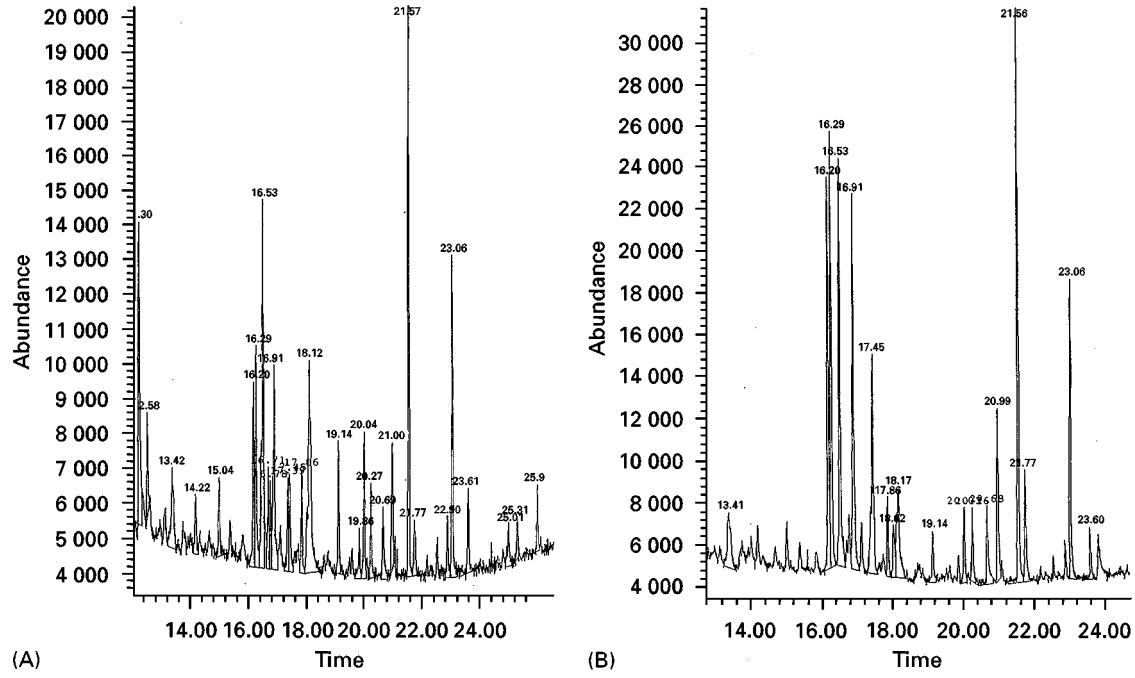

Figure 2 Chromatograms of samples removed from two works by William Blake: (A) white priming sample from *Spiritual Form of Nelson Guiding Leviathan* (1805–9); (B) green paint sample from *Body of Christ Borne to the Tomb* (1799–1800).

in an attempt to protect them from weathering and contamination and, though not classed as media themselves, their characterization may provide important evidence when determining the reasons for the ageing/degradation of works of art.

Varnish samples are commonly saponified prior to methylation, then the components separated on a capillary column with a linear temperature programme. Using mass spectrometry as a detection technique, two major peak groups can be seen, corresponding to the diterpenoid and triterpenoid components.

Waxes are stable materials comprising hydrocarbons and esters and, because of the virtual absence of polar groups, they can be analysed directly by high temperature capillary GC without the need for derivatization.

Reversed-phase High Performance Liquid Chromatography

The speed and sensitivity of reversed-phase high performance liquid chromatography (RP-HPLC) has led to significant developments in the analysis of proteins: RP-HPLC is now one of the most widely used techniques for the analysis of amino acids, since pre-column derivatization is possible with a selection of derivatizing agents and a variety of detection techniques can be employed. RP-HPLC lends itself well to conservation science, being particularly suitable for the analysis of the extremely small samples removed from works of art.

Phenylthiocarbamyl derivatives of amino acids in the hydrolysate of proteinaceous media samples have been separated on a C_{18} column using a ternary solvent system as the mobile phase (water–acetonitrile–acetate buffer).

Following hydrolysis of proteinaceous material removed from a series of Italian 15th-century painted panels, Halpine used phenyl isothiocyanate (PITC) for the derivatization of the amino acids, which were then separated on a C_{18} column using a binary solvent system of acetonitrile and acetate buffer (**Table 2**). The addition of nor-leucine as an internal standard facilitated the quantification of the amino acid components in the proteins, which in turn resulted in the characterization of a number of animal glue and egg/glue media. However, PITC-amino acid derivatives degrade in solution, so must be stored at low temperature prior to analysis.

PITC derivatives have also been analysed by RP HPLC when attempting to identify media samples which had been removed from a variety of French and Italian stone and wooden sculptures, frescoes and statues. Proteinaceous material was extracted from the matrices with sodium hydroxide and the subsequent analysis indicated the presence of gelatine and egg proteins.

9-Fluorenylmethyl chloroformate (FMOC) is a useful derivatizing agent for amino acids since it favours mild, aqueous conditions, reacts with both primary and secondary amino acids and is stable at room

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Table 2 Percentage amino acid composition

	Percentage amino acid composition							
	Control samples				*Samples from panels*			
Amino acid	*Control 1*	*Control 2*	*Control 3*	*Control 4*	*Sample 1*	*Sample 2*	*Sample 3*	*Sample 4*
Aspartic acid	1.0	9.1	5.6	7.9	5.5	*	10.2	5.2
Glutamic acid	2.3	11.1	9.3	13.0	5.9	6.4	9.8	4.1
Hydroxyproline	12.3	0.6	0.3	—	11.7	9.9	3.8	5.9
Serine	3.8	11.1	10.7	9.4	5.7	5.7	8.4	8.9
Glycine	27.7	10.9	7.8	5.2	26.9	24.7	15.8	18.6
Histamine	0.8	1.3	1.5	1.6	*	*	*	1.2
Arginine	5.7	5.3	5.9	4.0	4.1	*	4.3	3.0
Threonine	2.6	5.6	6.6	3.9	3.9	*	4.8	6.6
Alanine	11.1	8.6	10.2	8.8	11.8	11.2	10.0	12.8
Proline	16.9	5.4	5.9	4.0	11.6	12.4	6.7	10.3
Tyrosine	1.2	2.7	3.6	2.4	*	*	1.1	1.8
Valine	3.3	6.6	7.8	7.5	4.2	5.3	5.5	7.2
Methionine	1.3	1.7	2.0	4.8	*	6.7	1.3	0.3
Isoleucine	1.9	4.3	5.0	5.8	3.6	11.7	4.7	4.6
Leucine	3.9	8.5	10.0	9.0	1.5	6.0	7.2	7.1
Phenylalanine	2.0	3.3	3.7	5.9	3.7	*	3.3	2.4
Lysine	2.0	4.0	4.5	5.7	*	*	3.0	*

*Too small to quantify; control 1, rabbit skin glue ground with blue pigment; control 2, egg yolk with red pigment; control 3, egg yolk with blue pigment; control 4, egg albumin; sample 1, light blue paint; sample 2, dark blue paint; sample 3, light brown paint; sample 4, green paint. All paint samples removed from Cosimo Tura's *The Annunciation with Saint Francis and Saint Louis of Toulouse* (*c.* 1475). Reproduced from Halpine (1992) with permission.

temperature for up to 2 weeks. The FMOC moiety is both highly fluorescent and a good UV chromophore, so absorption and emission detection techniques can be used. Detection methods are an important concern in conservation science in view of the very small samples available – FMOC is particularly useful since fluorescence is usually far more sensitive than UV absorption. Standard proteinaceous media and museum sample hydrolysates have been characterized as FMOC derivatives.

Other Chromatographic Techniques

Ion Exchange Chromatography

Ion exchange chromatography was first used for the analysis of samples from works of art in 1969 when the successful analysis of antique and modern art specimens was reported. The eluted amino acids were detected by optical density with ninhydrin, then the percentage amino acid composition was calculated for each sample. Samples of gelatine, casein, glair, tempera and even animal horn were characterized using this method.

The use of ion exchange chromatography in this particular area is problematic: the method of sample preparation is both lengthy and complex, pH gradients are difficult to control precisely and the required sample size of paint is relatively large when put into the context of a specimen to be removed from a valuable work of art. Furthermore, museums and galleries are notoriously short of both money and space, thus specific single-purpose equipment is deemed unaffordable by many institutions.

Thin-layer Chromatography

TLC has been used on many occasions, particularly in the analysis of natural gum media – it is often used in conjunction with GC for such analyses but can provide useful information when used alone.

TLC has been used to characterize gum media taken from a 16th-century manuscript: hydrolysed gum samples were separated on silica plates, facilitating the subsequent identification of gum arabic.

Samples of binding media from paint layers were removed from three ancient Egyptian epitaphal stelae on wooden supports, then TLC was used to investigate the nature of the media, revealing the presence of gum tragacanth.

Pyrolysis–Gas Chromatography

Pyrolysis–gas chromatography (Py-GC) has been employed in the analysis of natural gum media from works of art. The distinctive pyrograms obtained for a series of standard gum samples enabled their identification and it was discovered that by pyrolysing the

complex gum-pigment mixed samples at 400°C, differences in peak patterns between standard samples and mixed samples were minimized. Sample identification is aided by the use of computational methods of pattern recognition.

Conclusions

When preparing to analyse a sample removed from a work of art, conservation scientists must select a technique which gives the maximum amount of information for the minimum amount of sample and sample preparation: it appears that RP-HPLC using FMOC as the amino acid derivatizing agent is the optimum analytical technique for the characterization of proteinaceous binding media, whilst GC is routinely used for oil-based media.

At present, GC analysis of silylated sugar residues is arguably the best method for the identification of natural gum media, and the use of mass spectrometry as the detection technique offers superior sensitivity and flexibility for these complex samples. However, this seems to be the least investigated area of analysis and significant developments in methodology which will further improve the sensitivity of the technique can be anticipated: this is of particular importance in the analysis of gum media since samples invariably contain no more than 10% binding medium, resulting in minute amounts of actual analyte in the samples. It is possible that microbore HPLC techniques may find a use in conservation science, since they are obviously suited to the minuscule samples routinely provided for analysis.

Simple qualitative techniques such as TLC may be sufficient to indicate the basic media type used in works of art, but as more and more works require some form of conservation or restoration treatment it is becoming increasingly important that the conservator has as much information as possible relating to the nature of the materials used in the work, in order to avoid damaging irreplaceable objects of artistic importance.

Chromatographic techniques provide reliable and accurate methods of analysis, suitable for use with the microscopic samples typically seen in this field of work. Further work should lead to simplification of methods of sample preparation – any improvements which mean that the size of samples required for analysis is reduced and that analyte losses are minimized would be welcomed by the conservation community.

See Colour Plates 59, 60.

See also: **II/Chromatography: Gas:** Derivatization; **Chromatography: Liquid:** Derivatization. **III/Amino Acids:** Gas Chromatography; Liquid Chromatography; Thin-Layer (Planar) Chromatography. **Paints and Coatings: Pyrolysis:** Gas Chromatography. **Pigments:** Liquid Chromatography; Thin-Layer (Planar) Chromatography. **Polysaccharides:** Liquid Chromatography.

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